



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2017

MEK inhibition and immune responses in advanced melanoma

Dummer, Reinhard ; Ramelyte, Egle ; Schindler, Sabrina ; Thürigen, Olaf ; Levesque, Mitchell P ;
Koelblinger, Peter

DOI: <https://doi.org/10.1080/2162402X.2017.1335843>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-139280>

Journal Article

Accepted Version

Originally published at:

Dummer, Reinhard; Ramelyte, Egle; Schindler, Sabrina; Thürigen, Olaf; Levesque, Mitchell P; Koelblinger, Peter (2017). MEK inhibition and immune responses in advanced melanoma. *OncoImmunology*, 6(8):e1335843.

DOI: <https://doi.org/10.1080/2162402X.2017.1335843>

MEK inhibition and immune responses in advanced melanoma

¹ Reinhard Dummer, ¹ Egle Ramelyte, ² Sabrina Schindler, ³ Olaf Thürigen, ² Mitchell P. Levesque, ¹ Peter Koelblinger

¹ University Hospital Zurich, Department Dermatology, Gloriastrasse 31, 8091 Zürich, reinhard.dummer@usz.ch, egle.ramelyte@usz.ch, peter.koelblinger@usz.ch

² University Hospital Zurich, Department Dermatology, Wagistrasse 14, 8952 Zürich, sabrinaannie.schindler@usz.ch, mitchell.levesque@usz.ch

³ Pierre Fabre Pharma GmbH, Jechtingerstrasse 13, D-79111 Freiburg, olaf.thuerigen@pierre-fabre.com

Corresponding Author:

Prof. Dr. med. Reinhard Dummer

Vice-Chairman University Hospital Zurich, Department Dermatology

Gloriastrasse 31

8091 Zürich

reinhard.dummer@usz.ch

Keywords:

BRAF mutation, immunotherapy, immunogenicity, Melanoma, MEK kinase pathway, MEK inhibitors, NRAS mutation

Funding details:

The University Zurich supported this work. Verein für Hautkrebsforschung, Euronco Stiftung (Ramelyte Egle), Peter Kölblinger, Sabrina Schindler

Abstract:

Phase II and III clinical trials demonstrated modest anti- tumor activity of Binimetinib (MEK162) - a potent allosteric inhibitor of MEK1 and MEK2- in patients with advanced NRAS mutant melanoma.

The analysis of the NEMO study in NRAS mutated melanoma, has shown that pre-treatment with immunotherapy improved the outcome of binimetinib therapy.

We discuss this finding in the context of *in vitro* and *in vivo* effects of MEK inhibition on immuno-critical pathways and interactions.

Introduction

Cutaneous melanomas are malignancies that originate from melanocytes, which derive from neural crest cells. Recently, the genetic and genomic landscapes of melanoma were investigated by next generation sequencing and have revealed several cancer-driving oncogenes. Most, if not all cutaneous melanomas present genetic alterations, resulting in an increased activity of the mitogen-activated protein kinase (MAPK) pathway, including mutations of BRAF, NRAS and RAC1. In addition, mutations can inactivate important tumor suppressor genes, such as neurofibromin 1 (NF1), ARID2, tumor protein p 53 (TP53) and cyclin-dependent kinase Inhibitor 2A (CDKN2A), some of which modulate MAPK pathway activity [1].

In vitro investigations have demonstrated that detection of the MAPK pathway activating *BRAF* or *NRAS* mutations, predicts the sensitivity to MEK inhibitors, which interfere with the activity of the kinases MEK1 and MEK2 [2] [3]. Consequently, MEK kinase inhibitors have been investigated in several clinical trials in metastatic cutaneous melanomas.

Clinical data

Already between July 2006 and June 2007, one of the first larger clinical trials in advanced melanoma compared the efficacy of orally administered selumetinib and temozolomide in chemotherapy-naïve patients. Selumetinib (AZD6244/ARRY-142886)

is an orally available, potent, selective, allosteric inhibitor of MEK1/2 with preclinical antitumor activity in melanoma (16). It was the first multicenter, randomized study conducted in patients with melanoma assessed for both *BRAF* and *NRAS* mutations. No significant difference in progression-free survival (PFS) was observed between selumetinib and temozolomide in patients unselected for *BRAF* or *NRAS* mutations. However, five out of six patients with partial response (PR) to selumetinib presented *BRAF* mutant tumors [4] suggesting that molecular profiling would help to identify patients who will benefit from this therapy.

This hypothesis was tested in a large phase III clinical trial investigating the MEK inhibitor (MEKi) trametinib in *BRAF*-mutated advanced melanoma in comparison to chemotherapy [5]. The outcome of this study was a median PFS of 4.8 months in the trametinib arm and 1.5 months in the chemotherapy arm. The superiority of MEKi was reflected in the hazard ratio (HR) of 0.45 (95% CI, 0.33 - 0.63; $p < 0.001$). The 6-month overall survival (OS) was 81% in the trametinib arm and 67% in the chemotherapy arm. The HR for death was 0.54 (95% CI, 0.32 - 0.92; $p < 0.014$) in the trametinib arm versus the chemotherapy arm, despite crossover which demonstrated the impressive efficacy of MEKi in *BRAF*-mutated melanoma.

Binimetinib (MEK162) is a potent, selective, non-adenosine triphosphate (ATP)-competitive allosteric inhibitor of MEK1 and MEK2. It has demonstrated growth

inhibition of *NRAS*- and *BRAF*^{V600E}-mutant melanoma in various preclinical models *in vitro* and *in vivo*. Binimetinib was investigated in a phase II clinical trial, that enrolled 30 patients with *NRAS*-Q61-mutated and 41 patients with *BRAF*-mutated melanoma into the 45 mg arm [6]. Pharmacokinetic profiling revealed that binimetinib is well absorbed, with a median T_{max} of 1.48 hours on day 15 with moderate interpatient variability. Partial response (PR) was observed in 6/30 (20% [95% CI, 8–39]) of patients with *NRAS* mutations. The median response duration was approximately eight weeks.

The median PFS was 3.7 (95% CI, 2.5–5.4) months for patients with *NRAS*-mutated melanoma. This modest, but clinically meaningful effect in the *NRAS*-mutated advanced melanoma population was the rationale to develop a phase III clinical trial called NEMO (*NRAS* melanoma and *MEK* inhibitor) in this genetically defined patient population with a high medical need. All the more so after the failure of immunotherapy, which is the preferred 1st line treatment option in patients with metastatic *NRAS*-mutated melanoma today [7].

The NEMO protocol is a randomized phase III, open-label, multicenter, two-arm clinical trial, comparing binimetinib to dacarbazine in patients with advanced unresectable or metastatic melanoma harboring *NRAS* mutations. Stratification factors included performance status, stage and prior immunotherapy.

Between July 2013 and April 2015, 402 patients were enrolled, 269 of which were randomized to binimetinib and 133 to a dacarbazine-arm. Binimetinib achieved a superior

PFS (HR 0.62, 95% CI, 0.47 – 0.80; $p < 0.001$). Median PFS was 2.8 months (95% CI, 2.8 – 3.6) for binimetinib and 1.5 months (95% CI, 1.5 – 1.7) for dacarbazine. Median OS was 11.0 months (8.9–13.6) for binimetinib and 10.1 months (95% CI, 7.0 – 16.5) for dacarbazine (HR 1.00 [0.75 – 1.33]; $p = 0.499$).

Binimetinib treatment was associated with a higher confirmed overall response rate (ORR) compared with dacarbazine (15.2% [95% CI, 11.2% – 20.1%; $n = 41$] vs 6.8% [95% CI, 3.1% – 12.5%; $n = 9$]; $p = 0.015$, two-sided test). Four patients (1.5%) in the binimetinib arm and no patients in the dacarbazine arm achieved a complete response (CR). Additionally, binimetinib achieved a higher disease control rate than dacarbazine with 58.4% in the binimetinib arm vs 24.8% in the dacarbazine arm.

PFS in most pre-specified patient subgroups was consistent with the overall population, including high risk subgroups such as patients with elevated lactate dehydrogenase (LDH) serum levels or advanced disease stage (M1c). In the population of patients pretreated with immunotherapy, median PFS was longer for those who received binimetinib vs dacarbazine (5.5 months vs 1.6 months). Further, within this stratum, the confirmed ORR per central review was 15.8% vs 3.6%, and the median duration of response was 11.1 months vs 4.1 months, in the binimetinib vs dacarbazine arms, respectively (Dummer R. et al. Binimetinib versus dacarbazine in patients with advanced *NRAS*-mutant melanoma (NEMO): a multicentre, open-label,

randomised, phase 3 trial_Lancet Oncology Published online March 8, 2017). Since nowadays most advanced melanoma patients receive immunotherapy as 1st line treatment, these data are relevant for the management of this patient population. In order to understand these clinical observations, it is reasonable to review preclinical investigations.

Preclinical data

In a recently published report [8] by Brea et al., it was convincingly demonstrated, that the inhibition of an over activated MAPK pathway by MEKi or EGFR inhibitors results in the up-regulation of HLA class I molecules in several tumor cell lines. This effect was further enhanced in presence of interferon-gamma. It is accompanied by an upregulation of key components of the antigen processing machinery including TAP transporters and beta2 microglobulin at the mRNA level.

These data are consistent with the results reported by Liu et al. (2015) [9] who investigated *BRAF* mutant melanoma cells treated with *BRAF* inhibitors (BRAFi), MEKi or both for a period of 6 hours up to two days. They documented an increase in HLA class I and II, including non-classical HLA-E expression, an enhanced expression of immunomodulating molecules including CD40, CD68, CD70, CD83, GBP1, ICOSLG, IL15, IRF1, OX40L, SPP1, STAT1, STAT3, TOX, B7-H3, PDCD2, and a decrease in

expression of immunosuppressive factors such as IL1A, IL8, NT5E, VEGFA and PDL1 along with a series of other effects on molecules involved in apoptosis.

Long-term exposure (2 weeks) to a MEKi or a pan-BRAFi, exerts different effects depending on the phenotype of the tumor cells, as described by Zipser et al [10]. This work demonstrates a phenotype switch in MITF expressing tumor cells with an activated MAPK pathway, characterized by a change in morphology, increased invasiveness and a reduced expression of melanocytic differentiation antigens [10]. The immunological consequences of these alterations remain unclear. We assume that immunogenicity might be reduced after long-term exposure in contrast to short-term. Deken et al. [11] who investigated the impact of the MAPK pathway in a mouse model reported an influx of T-cells during the 1st week of therapy with a BRAFi alone, and a lesser presence of T-cells later on, supporting our hypothesis. Intermittent pulsing of kinase inhibitors might be helpful to overcome this problem [12].

MEKi effect on T-cells

Given the central role of the MAPK pathway in T-cell receptor signaling, concerns were rapidly raised regarding the effect of MEKi on T-cell functions [13] [14] . In 2010, Boni et al. showed that unlike BRAFi, MEKi impairs T-cell functions *in vitro*. They reported a decrease in proliferation and viability, as well as a diminished response against melanoma

cells when the cultures were grown in presence of MEKi [15] . Further *in vitro* investigation on the effect of MEKi on the different T-cell sub-populations revealed a decrease in proliferation in CD8⁺ and CD4⁺ T-cells and a concentration-dependent decline in the generation of antigen-specific T lymphocytes [16]. The investigation of the effect of MEKi on the different T-cell stages revealed a more complex and context-dependent regulation.

In 2015, Liu et al. [9] showed contrasts in response depending on the sequence of T-cell activation and MEKi treatment. When CD4⁺ T-cells are dosed with MEKi 24h before activation (at clinically relevant concentrations), a transient decrease in proliferation can be seen after 3 days of treatment; this effect however dissipates at day 7 [9] . MEK inhibition was also reported to limit CD4⁺ T-cell activation induced apoptosis.

As opposed to what has been previously observed *in vitro*, different groups demonstrated that the cytotoxic capabilities of T-cells extracted from tumor bearing mice treated with MEKi, are not impaired and can respond to re-stimulation *ex-vivo* by releasing IFN γ [17]

Since complex interactions between tumor cells and lymphocytes cannot be assessed *in vitro*, further studies were carried out *in vivo*. In that context, it was clearly established that MEKi treatment alone or in combination to BRAFi increased CD8⁺ tumor infiltration in mice [11,17] . Not only was CD8⁺ T-cell function under MEKi treatment shown not to be impeded, but it also seemed to be indispensable for an optimal anti-tumor response.

Different groups showed that depletion of CD8⁺ does not attenuate MEKi treatment efficiency [18,19].

A more recent study from Ebert et al shed more light on the specific action of MEKi, *in vivo*, on different T-cell stages in mice [19]. They first confirmed the activity of MEKi at the tumor site by showing a reduction in pERK. Their data corroborated a higher CD8⁺ influx into the tumor upon MEKi treatment, but also illustrated phenotypic differences compared to control (vehicle treated) tumor-infiltrating lymphocytes (TILs). TILs from MEKi treated mice had a lower ratio of PD-1^{hi}/PD-1^{low} expression compared with control and expressed higher levels of T-bet and Eomes, 2 transcription factors controlling CD8⁺ T-cell differentiation towards effector functions. However, the analysis of the lymph nodes revealed a different behavior of T-cells upon MEKi treatment before priming.

Indeed, the treatment prevented the expansion of naïve CD8⁺ and the up-regulation of T-bet in response to stimulation, but did not deplete them.

As described for CD4⁺ T-cells, MEKi seems to prevent activation-induced apoptosis in CD8⁺ T-cells. Antigen-experienced T-bet^{hi} CD8⁺ T-cells have been shown to be particularly vulnerable to this fate (via up-regulation of Nur77 and caspase activation) but the addition of MEKi leads to an accumulation of those tumor-specific CD8⁺ T-cells.

Finally, the authors proved that this enriched pool of antigen-specific T-cells is also capable of killing target cells and that MEKi treatment does not affect this process.

Inflammatory skin reactions are frequent in patients treated with MEKi [4,20]. In the normal epidermis, active EGFR mediated MAPK pathway activation is mainly seen in the basal keratinocytes. The sudden interruption of this signaling pathway by MEKi results in an acute keratinocyte stress response with an upregulation of *p53* including a disturbed epidermal homeostasis associated with inflammation and tissue damage caused by the influx of neutrophils and lymphocytes. Similar changes of the microenvironment might also occur in the tumor microenvironment

Discussion: Comparison to BRAFi in *BRAF*-mutated melanoma

As outlined above, MEKi treatment confers distinct effects on melanoma cells that influence tumor cell immunogenicity, microenvironment, and the host immune responses via interference with the MAPK pathway in various cell types. Before the advent of MEKi in large-scale clinical trials, selective inhibitors of the BRAF molecule had already been shown to induce robust tumor responses in patients with *BRAF*-mutated metastatic melanoma [21,22]. The antitumor effect of BRAFi was primarily attributed to the direct blockade of MAP kinase signaling. Subsequently, evidence has emerged that BRAF inhibition additionally elicits various immunological changes affecting the tumor-host interaction, which may also contribute to the beneficial effects of BRAFi in the *BRAF*-mutant patient population. In *BRAF*-mutated tumor cells, the immunolo-modulatory effects induced by BRAFi are to some extent in line with those described for MEKi above. These

effects have been demonstrated both *in vitro* and *in vivo* and include an increased expression of melanoma-associated antigens, a decreased release of immunosuppressive cytokines (IL6, IL8, IL-1, VEGF) by the tumor as well as an enhanced tumor infiltration by CD4⁺ and CD8⁺ TILs [23-25]. However, in *BRAF*-wild type cells, BRAFi induce contrasting immunological modifications, mostly due to the paradoxical activation of the MAPK pathway which exclusively occurs with BRAFi treatment, in a CRAF-dependent manner [26]. This mechanism is thought to further enhance effector function of T- or NK-cells by inducing oligoclonal expansion [27]. On the other hand, paradoxical MAPK pathway activation has been associated with an increased expression of T-cell exhaustion markers such as PD-1 and TIM3 on T-cells as well as PD-L1 on tumor cells, which attenuates immune response [23]. Moreover, it has been suggested that paradoxical MAPK pathway activation may increase autoimmunity and frequency of immune-related adverse events by over-activating T-cells, especially when combining BRAFi with immunotherapy, and, conversely, may lead to enhanced function of regulatory immune cells such as myeloid-derived suppressor cells or T regulatory cells. Intriguingly, these negative immune effects of BRAFi monotherapy are partially reversed by MEK-inhibition. That is, increased surface expression of PD-L1 in BRAFi resistant melanoma cell lines is diminished after treatment with a selective MEKi [28]. In a more clinical context, tumor samples from patients treated with BRAFi monotherapy showed, after an initial increase in the first weeks of treatment, a decrease in melanoma-associated antigen expression that could reflect the phenotype switch *in vivo* and reduced T-cell infiltration at the time of disease progression.

These changes are subsequently reverted by the addition of a MEKi [23]. As discussed earlier in this review, the recently described potentially protective effect of MEKi on antigen-experienced CD8⁺ cells may as well contribute to this clinical phenomenon [19].

Taken together, these findings give a strong rationale to combine MAPK-pathway inhibitors (either BRAF-/MEKi combination in BRAF-mutated tumors or single agent MEK-I in BRAF Wt tumors) with immunotherapy. Randomized clinical trials investigating the addition of PD1-/PDL1-antibodies to targeted therapy have been initiated. Early clinical results of these combinations demonstrated feasibility and tolerability in conjunction with an impressive anti-tumor efficacy (Sullivan R et al, oral presentation SMR Congress 2016; Infante J et al, oral presentation SMR Congress 2016. ClinicalTrials.gov Identifier: NCT02908672; ClinicalTrials.gov Identifier: NCT02130466).

Conclusion

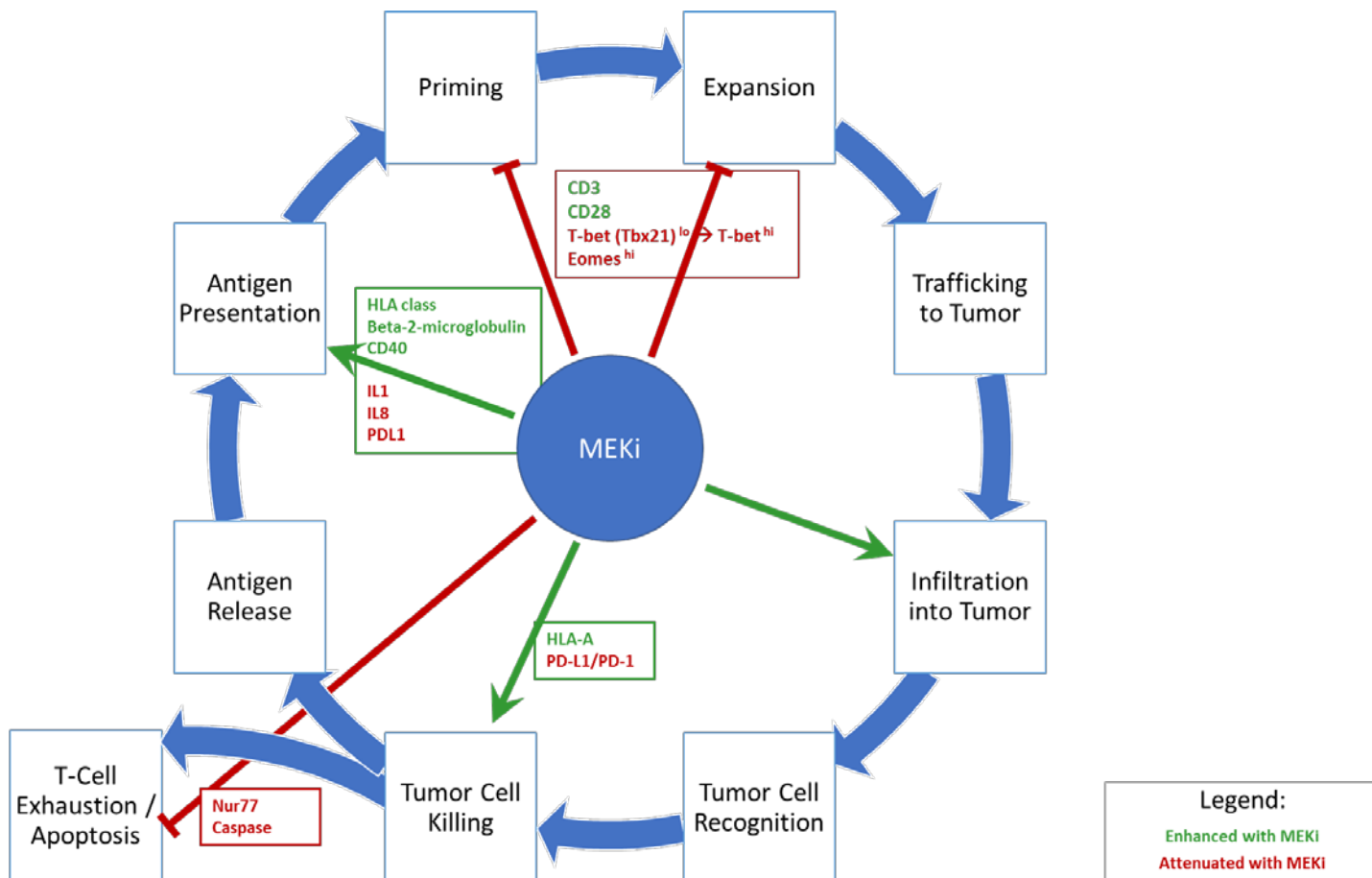
The mechanisms of immunotherapies using checkpoint inhibitors are complex. They typically result in the activation of primed T-cells *in vivo*, however, in a chronically inflamed environment such as the tumor, the T-cell are prone to exhaustion and apoptosis. Addition of MEKi might reduce this risk.

Moreover, MEKi increases the immunogenicity of melanoma cells by upregulating immuno-critical molecules including HLA class I, molecules involved in antigen processing and cancer antigens, but also reduce suppressive factors such as PD-L1 on

tumor cells or immunosuppressive cytokines, at least during the early treatment phase. In addition, there is evidence that access of inflammatory cells to the tumor microenvironment may be improved in the presence of MEKi.

These findings may explain the differences observed in MEKi monotherapy in immunotherapy naïve versus pretreated patients and provide a strong rationale to develop MEKi/immunotherapy combination strategies. Intermittent kinase inhibitor therapy deserves specific attention in this context.

Figure 1: MEKi may have negative impact on T cell priming but may enhance the survival of activated T cells. They can increase cancer antigen presentation and immunogenicity of tumor cells. (modified after Chen DS & Mellman I, Immunity 2013, 33).



References

- 1 Krauthammer M, Kong Y, Bacchiocchi A, Evans P, Pornputtapong N, Wu C, McCusker JP, Ma S, Cheng E, Straub R, Serin M, Bosenberg M, Ariyan S, Narayan D, Sznol M, Kluger HM, Mane S, Schlessinger J, Lifton RP, Halaban R: Exome sequencing identifies recurrent mutations in NF1 and RASopathy genes in sun-exposed melanomas. *Nat Genet* 2015
- 2 Raaijmakers MI, Widmer DS, Narechania A, Eichhoff O, Freiburger SN, Wenzina J, Cheng PF, Mihic-Probst D, Desalle R, Dummer R, Levesque MP: Co-existence of BRAF and NRAS driver mutations in the same melanoma cells results in heterogeneity of targeted therapy resistance. *Oncotarget* 2016;7:77163-77174.
- 3 Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, Ye Q, Lobo JM, She Y, Osman I, Golub TR, Sebolt-Leopold J, Sellers WR, Rosen N: BRAF mutation predicts sensitivity to MEK inhibition. *Nature* 2006;439:358-362.
- 4 Kirkwood JM, Bastholt L, Robert C, Sosman J, Larkin J, Hersey P, Middleton M, Cantarini M, Zazulina V, Kemsley K, Dummer R: Phase II, open-label, randomized trial of the MEK1/2 inhibitor selumetinib as monotherapy versus temozolomide in patients with advanced melanoma. *Clin Cancer Res* 2012;18:555-567.
- 5 Flaherty KT, Robert C, Hersey P, Nathan P, Garbe C, Milhem M, Demidov LV, Hassel JC, Rutkowski P, Mohr P, Dummer R, Trefzer U, Larkin JM, Utikal J, Dreno B, Nyakas M, Middleton MR, Becker JC, Casey M, Sherman LJ, Wu FS, Ouellet D, Martin

- AM, Patel K, Schadendorf D: Improved survival with MEK inhibition in BRAF-mutated melanoma. *N Engl J Med* 2012;367:107-114.
- 6 Ascierto PA, Schadendorf D, Berking C, Agarwala SS, van Herpen CM, Queirolo P, Blank CU, Hauschild A, Beck JT, St-Pierre A, Niazi F, Wandel S, Peters M, Zuber A, Dummer R: MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study. *Lancet Oncol* 2013;14:249-256.
 - 7 Dummer R, Hauschild A, Lindenblatt N, Pentheroudakis G, Keilholz U: Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015;26 Suppl 5:v126-132.
 - 8 Brea EJ, Oh CY, Manchado E, Budhu S, Gejman RS, Mo G, Mondello P, Han JE, Jarvis CA, Ulmert D, Xiang Q, Chang AY, Garippa RJ, Merghoub T, Wolchok JD, Rosen N, Lowe SW, Scheinberg DA: Kinase Regulation of Human MHC Class I Molecule Expression on Cancer Cells. *Cancer Immunol Res* 2016;4:936-947.
 - 9 Liu L, Mayes PA, Eastman S, Shi H, Yadavilli S, Zhang T, Yang J, Seestaller-Wehr L, Zhang SY, Hopson C, Tsvetkov L, Jing J, Zhang S, Smothers J, Hoos A: The BRAF and MEK Inhibitors Dabrafenib and Trametinib: Effects on Immune Function and in Combination with Immunomodulatory Antibodies Targeting PD-1, PD-L1, and CTLA-4. *Clin Cancer Res* 2015;21:1639-1651.
 - 10 Zipser MC, Eichhoff OM, Widmer DS, Schlegel NC, Schoenewolf NL, Stuart D, Liu W, Gardner H, Smith PD, Nuciforo P, Dummer R, Hoek KS: A proliferative melanoma cell phenotype is responsive to RAF/MEK inhibition independent of BRAF mutation status. *Pigment Cell Melanoma Res* 2011;24:326-333.
 - 11 Deken MA, Gadiot J, Jordanova ES, Lacroix R, van Gool M, Kroon P, Pineda C, Geukes Foppen MH, Scolyer R, Song JY, Verbrugge I, Hoeller C, Dummer R, Haanen JB, Long GV, Blank CU: Targeting the MAPK and PI3K pathways in combination with PD1 blockade in melanoma. *Oncoimmunology* 2016;5:e1238557.
 - 12 Das Thakur M, Salangsang F, Landman AS, Sellers WR, Pryer NK, Levesque MP, Dummer R, McMahon M, Stuart DD: Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature* 2013;494:251-255.
 - 13 Sharp LL, Schwarz DA, Bott CM, Marshall CJ, Hedrick SM: The influence of the MAPK pathway on T cell lineage commitment. *Immunity* 1997;7:609-618.
 - 14 D'Souza WN, Chang CF, Fischer AM, Li M, Hedrick SM: The Erk2 MAPK regulates CD8 T cell proliferation and survival. *J Immunol* 2008;181:7617-7629.
 - 15 Boni A, Cogdill AP, Dang P, Udayakumar D, Njauw CN, Sloss CM, Ferrone CR, Flaherty KT, Lawrence DP, Fisher DE, Tsao H, Wargo JA: Selective BRAFV600E inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function. *Cancer Res* 2010;70:5213-5219.
 - 16 Vella LJ, Andrews MC, Pasam A, Woods K, Behren A, Cebon JS: The kinase inhibitors dabrafenib and trametinib affect isolated immune cell populations. *Oncoimmunology* 2014;3:e946367.
 - 17 Hu-Lieskovan S, Mok S, Homet Moreno B, Tsoi J, Robert L, Goedert L, Pinheiro EM, Koya RC, Graeber TG, Comin-Anduix B, Ribas A: Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma. *Sci Transl Med* 2015;7:279ra241.

- 18 Allegrezza MJ, Rutkowski MR, Stephen TL, Svoronos N, Perales-Puchalt A, Nguyen JM, Payne KK, Singhal S, Eruslanov EB, Tchou J, Conejo-Garcia JR: Trametinib Drives T-cell-Dependent Control of KRAS-Mutated Tumors by Inhibiting Pathological Myelopoiesis. *Cancer Res* 2016;76:6253-6265.
- 19 Ebert PJ, Cheung J, Yang Y, McNamara E, Hong R, Moskalenko M, Gould SE, Maecker H, Irving BA, Kim JM, Belvin M, Mellman I: MAP Kinase Inhibition Promotes T Cell and Anti-tumor Activity in Combination with PD-L1 Checkpoint Blockade. *Immunity* 2016;44:609-621.
- 20 Schad K, Baumann Conzett K, Zipser MC, Enderlin V, Kamarashev J, French LE, Dummer R: Mitogen-activated protein/extracellular signal-regulated kinase kinase inhibition results in biphasic alteration of epidermal homeostasis with keratinocytic apoptosis and pigmentation disorders. *Clin Cancer Res* 2010;16:1058-1064.
- 21 Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA: Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011;364:2507-2516.
- 22 Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, Millward M, Rutkowski P, Blank CU, Miller WH, Jr., Kaempgen E, Martin-Algarra S, Karaszewska B, Mauch C, Chiarion-Sileni V, Martin AM, Swann S, Haney P, Mirakhur B, Guckert ME, Goodman V, Chapman PB: Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 2012;380:358-365.
- 23 Frederick DT, Piris A, Cogdill AP, Cooper ZA, Lezcano C, Ferrone CR, Mitra D, Boni A, Newton LP, Liu C, Peng W, Sullivan RJ, Lawrence DP, Hodi FS, Overwijk WW, Lizee G, Murphy GF, Hwu P, Flaherty KT, Fisher DE, Wargo JA: BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin Cancer Res* 2013;19:1225-1231.
- 24 Wilmott JS, Long GV, Howle JR, Haydu LE, Sharma RN, Thompson JF, Kefford RF, Hersey P, Scolyer RA: Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. *Clin Cancer Res* 2012;18:1386-1394.
- 25 Whipple CA, Boni A, Fisher JL, Hampton TH, Tsongalis GJ, Mellinger DL, Yan S, Tafe LJ, Brinckerhoff CE, Turk MJ, Mullins DW, Fadul CE, Ernstoff MS: The mitogen-activated protein kinase pathway plays a critical role in regulating immunological properties of BRAF mutant cutaneous melanoma cells. *Melanoma Res* 2016;26:223-235.
- 26 Gibney GT, Messina JL, Fedorenko IV, Sondak VK, Smalley KS: Paradoxical oncogenesis--the long-term effects of BRAF inhibition in melanoma. *Nat Rev Clin Oncol* 2013;10:390-399.
- 27 Hu-Lieskovan S, Robert L, Homet Moreno B, Ribas A: Combining targeted therapy with immunotherapy in BRAF-mutant melanoma: promise and challenges. *J Clin Oncol* 2014;32:2248-2254.

28 Jiang X, Zhou J, Giobbie-Hurder A, Wargo J, Hodi FS: The activation of MAPK in melanoma cells resistant to BRAF inhibition promotes PD-L1 expression that is reversible by MEK and PI3K inhibition. Clin Cancer Res 2013;19:598-609.

Page 6: Dummer R. et al. Binimetinib versus dacarbazine in patients with advanced *NRAS*-mutant melanoma (NEMO): a multicentre, open-label, randomised, phase 3 trial_Lancet Oncology Published online March 8, 2017 [http://dx.doi.org/10.1016/S1470-2045\(17\)30180-8](http://dx.doi.org/10.1016/S1470-2045(17)30180-8)

Page 13: Sullivan R et al, Hamid O, Gonzalez R, Infante J et al. Safety and Clinical Activity of Atezolizumab+ Cobimetinib+ Vemurafenib in BRAFV600 Mutant Metastatic Melanoma. Oral presentation, Society of Melanoma Research Congress, Nov. 2016.

Infante J, Kim TM, Friedmann J, Miller WH et al. Safety and Clinical Activity of Atezolizumab Combined With Cobimetinib in Metastatic Melanoma. Oral presentation, Society of Melanoma Research Congress, Nov. 2016.